

Dioecy Derived from Distyly in *Morinda villosa* Hook. f. (*Rubiaceae*) Occurring in Hukaung Valley, Kachin State, Myanmar

Takashi SUGAWARA^{a,*}, Nobuyuki TANAKA^b and Jin MURATA^c

^aMakino Herbarium, Graduate School of Science, Tokyo Metropolitan University,
1-1, Minami-Ohsawa, Hachioji, Tokyo, 192-0397 JAPAN;

^bKochi Prefectural Makino Botanical Garden, 4200-6, Godaisan, Kochi, 781-8125 JAPAN;

^cBotanical Gardens, Graduate School of Science, The University of Tokyo,
3-7-1, Hakusan, Bunkyo-ku, Tokyo, 112-0001 JAPAN

*Corresponding author: sugakei@tmu.ac.jp

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Floral characters were examined to determine sexual system in the flowers of *Morinda villosa* Hook. f., a perennial woody climber occurring in Kachin State, Myanmar. The flowers are dimorphic, being either long-styled or short-styled morphs. The short-styled morph produced stainable pollen grains, but its style did not develop stigmatic papillae on the stigma. The long-styled morph, on the other hand, developed stigmatic papillae on the stigma, but did not produce pollen grains in its anthers. Only the long-styled plants produced seeds. Based on these results, we considered that this species was morphologically distylous but functionally dioecious, and thus its sexual system may be derived from distyly.

Key words: dioecy, distyly, *Morinda villosa*, Myanmar, *Rubiaceae*, sexual system.

Morinda L. (*Rubiaceae*), a large genus comprising more than 80 species, is trees, shrubs, or woody climbers, and mainly distributed in the Old World tropics and subtropics (Peterson 1990, Johansson 1994, Mabberley 2008). Among the species examined of *Morinda*, a wide range of sexual systems, including monomorphic hermaphrodite, distylous hermaphrodite, dioecy, and androdioecy, has been reported (Philip and Mathew 1978, Reddy and Bahadur 1978, Johansson 1994, Puff et al. 2005, Nishide et al. 2009, Sugawara et al. 2010). However, the sexual system in most of the *Morinda* species still remains unclear.

Morinda villosa Hook. f., which is one of the *Morinda* species distributed from southwestern

China (Yunnan) to Vietnam and northern India, has been regarded as monomorphic in floral morphology (Ruan 1999, Chen 2003). In our preliminary investigations, however, the species appeared to be dimorphic with a distylous nature and functionally dioecious. Distylous species are morphologically characterized by having two types of plants that bear different flower morphs: long- and short-styled morphs (Darwin 1877, Barrett and Richards 1990, Barrett 1992). In some distylous genera (e.g., *Cordia*, *Coussarea*, *Mussaenda*, *Nymphoides*, *Psychotria*, *Sarcotheca*, etc.), it is reported that distyly has evolved into dioecy (Opler et al. 1975, Bawa 1980, Beach and Bawa 1980, Barrett and Richards 1990, Webb 1999), and in

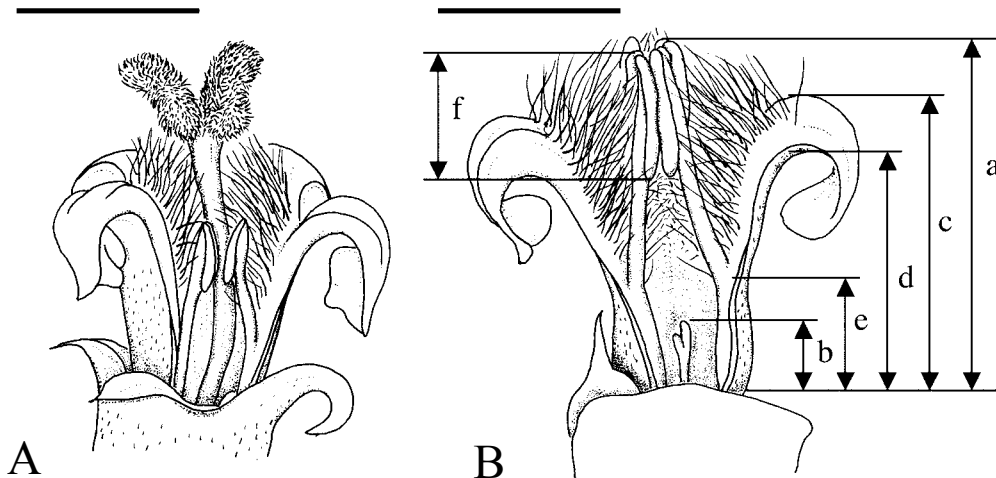


Fig. 1. Drawings of long- and short-styled morphs of *Morinda villosa*. A. Long-styled flower. B. Short-styled flower. a. Anther height. b. Stigma height. c. Corolla height-1. d. Corolla height-2. e. Corolla tube length. f. Anther length. All scale bars = 2 mm.

every case female plants are derived from long-styled morph, while male plants are derived from short-styled morph. Among the species examined of *Morinda*, the evolutionary change from distyly to dioecy has never been reported so far. In this paper, we report that *M. villosa* is morphologically distylous, but functionally dioecious, and that its sexual system may be derived from a distylous ancestor.

Materials and Methods

Morinda villosa is a climbing shrub morphologically characterized by elliptic leaves pubescent on both surfaces and camptodromous with 10 to 13 pairs of lateral veins (Hooker 1882, Ruan 1999, Chen 2003). Several years ago, seeds of *M. villosa* were collected along the Ledo Road, south of Tanaing (26°06'34"N 96°42'58"E, ca. 250 m alt., 19 Sept. 2005), Hukaung Valley, Kachin State, Myanmar (MBK 040414), and they were sown in pots at the Kochi Prefectural Makino Botanical Garden, Kochi, Japan. Next year, some of them germinated, and after five years several individuals opened flowers in the green house. In the present study we examined seven

individuals. Voucher specimens (N. Tanaka 040414R) were deposited in herbarium of the Kochi Prefectural Makino Botanical Garden (MBK), Kochi, and in Makino Herbarium (MAK), Tokyo Metropolitan University, Tokyo.

To observe morphological differentiation, several inflorescences with flowers and flower buds were randomly collected from seven plants and preserved in 70% ethanol. We measured six floral characters for each flower (Fig. 1): a) anther height, b) stigma height, c) corolla height from the base to the upper surface of corolla-lobe (corolla height-1), d) corolla height from the base to the lower surface of corolla-lobe (corolla height-2), e) corolla-tube length, and f) anther length.

Pollen stainability with aniline blue in lactophenol of more than 200 pollen grains per flower was examined for 19 flowers from seven plants.

Flowers and their parts were observed using scanning electron microscopy (SEM). The samples from a few plants were dehydrated in an ethanol: t-butanol series, freeze-dried using a freeze drying device (JFD-300, JEOL9, mounted onto SEM stubs on double-sided carbon tape,

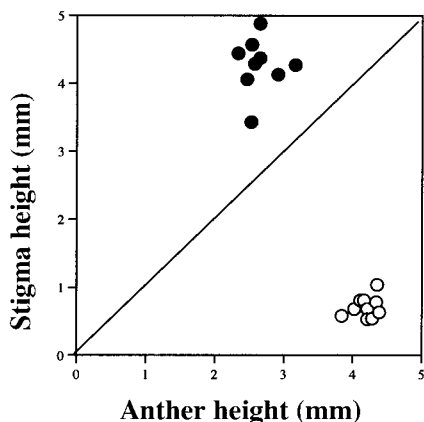


Fig. 2. Scatter diagram showing a relationship of stigma height and anther height in *Morinda villosa*. ●: Long-styled morph. ○: Short-styled morph.

coated with gold using an ion sputter (JFC-110E, JEOL) and observed using a scanning electron microscopy (JSM-5600LV, JEOL).

Observations and Discussion

Morinda villosa flowered from late May to June in Botanical Garden, Kochi Pref., Japan. Flowers were radially symmetrical with corolla. Corolla tubes were short, about 2 mm long, and their upper portion had many oblique-facing hairs on their inner surface (Fig. 1).

Morphological observations of *Morinda villosa* revealed the presence of two distinct floral morphs: long-styled morph with short

stamens and short-styled morph with long stamens (Fig. 2). In the long-styled morph, a style was extruded from the corolla tube and bifurcated, forming a stigma which develop long stigmatic papillae, while stamens remain within a corolla tube and their anthers are wholly concealed with a number of long, oblique-facing hairs (Fig. 3A, C). In the short-styled morph, on the other hand, stamens with long filaments were extruded from the corolla tube and their anthers dehiscence introrsely. A short style was positioned below the anthers and its stigma did not develop stigmatic papillae (Fig. 3B, D). Morphologically, the two morphs furthermore differed significantly from each other in corolla height and anther length (Table 1). However, no significant difference was found between the two morphs in corolla-tube length (Table 1).

Comparison of the stigma and anther heights from long- and short-styled flowers is shown in Figure 4. In the long-styled flowers, stigmas were significantly higher than the anthers. In the short-styled flowers, on the other hand, stamens were significantly higher than the stigmas. Anther heights of long-styled flowers significantly differed from stigma heights of short-styled flowers. In contrast, anther heights of short-styled flowers were not significantly different from stigma heights of long-styled flowers. These results indicate that although distylous flowers are not exactly reciprocal,

Table 1. Comparison of several floral characters between long- and short-styled morphs in *Morinda villosa*. N: number of plants examined

Character	Long-styled morph Mean \pm S. D.	Short-styled morph Mean \pm S. D.	Statistics (Mann-Whitney's U test)
No. of flowers examined (N)	9 (2)	10 (5)	
Anther height (mm)	2.64 \pm 0.25	4.19 \pm 0.17	$P < 0.001$
Stigma height (mm)	4.27 \pm 0.40	0.71 \pm 0.15	$P < 0.001$
Corolla height-1 (mm)	2.94 \pm 0.24	3.44 \pm 0.28	$P < 0.01$
Corolla height-2 (mm)	2.43 \pm 0.20	3.07 \pm 0.30	$P < 0.001$
Corolla-tube length (mm)	1.30 \pm 0.20	1.46 \pm 0.22	$P = 0.19$
Anther length (mm)	0.86 \pm 0.03	1.51 \pm 0.09	$P < 0.001$
Pollen stainability (%)	no pollen	95.66 \pm 1.67	

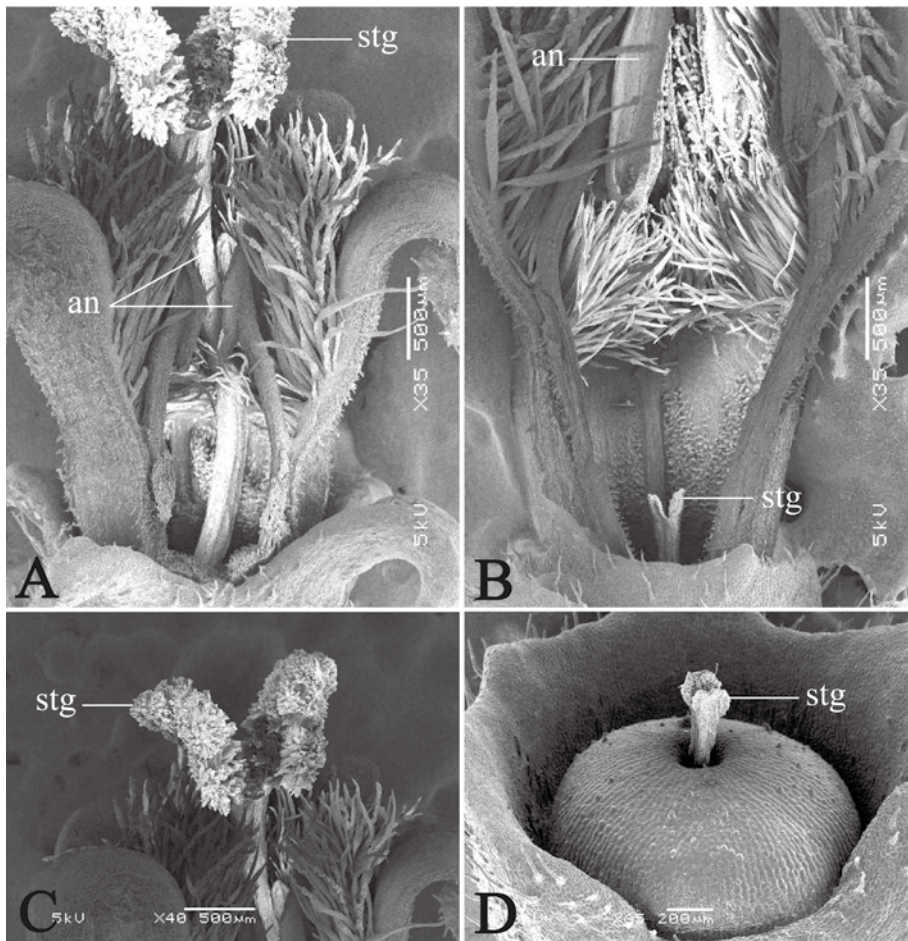


Fig. 3. SEM micrographs of long- and short-styled flowers of *Morinda villosa*. A. Long-styled flower showing a long style and two abortive stamens. B. Short-styled flower showing stamens with pollen grains and a short abortive style. C. Long-styled flower showing two stigmatic lobes which developed stigmatic papillae. D. Short-styled flower showing a short abortive style. stg. Stigma. an. Anther.

stigma and anther heights are reciprocal between long- and short-styled flowers.

Throughout flowering, the anthers of the long-styled flowers did not produce pollen grains, suggesting pollen sterility. Conversely, the anthers of the short-styled flowers produced a number of pollen grains, and their pollens stained well with aniline blue in lactophenol, with stainability exceeding 95% ($95.7 \pm 1.7\%$, $n = 10$). Although the ovaries of both short- and long-styled flowers consistently contained four ovules, mature fruits were observed only

on the long-styled flowers. Taken together, these findings indicate that *Morinda villosa* is functionally dioecious; that is, long-styled flowers are pistillate, and short-styled flowers staminate.

In the genus *Morinda*, dioecy is also found in *M. umbellata* L. subsp. *umbellata* distributed widely in southeastern Asia (Sugawara et al. 2010). Compared with this taxon, it is interesting to note that dioecious flowers of *M. villosa* retain a rudimentary short style as well as abortive stamens and are apparently distylous (see Fig.

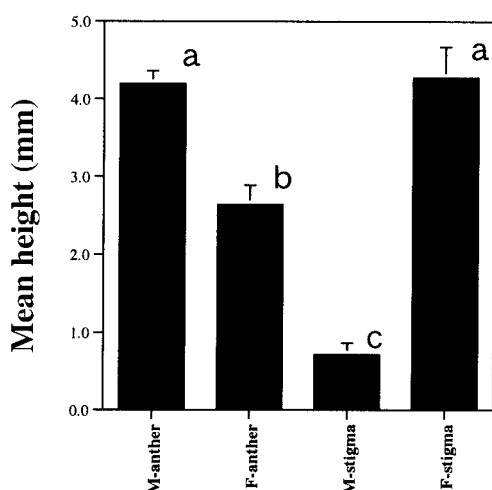


Fig. 4. Stigma and anther heights of long- and short-styled flowers of *Morinda villosa*. Vertical lines indicate standard deviations. Letters (a, b, and c) indicate significant differences at $p < 0.05$ determined by Steel-Dwass's multiple range test.

3). This floral trait suggests that the dioecious sexual system of *M. villosa* may be derived from a distylous ancestor, as documented in other genera, such as *Cordia* (*Boraginaceae*, Opler et al. 1975), *Coussarea* (*Rubiaceae*, Beach and Bawa 1980), *Nymphoides* (*Menyanthaceae*, Barrett and Richards 1990), *Mussaenda* (*Rubiaceae*, Naiki and Kato 1999), *Psychotria* (*Rubiaceae*, Sohmer 1977), and others (Bawa 1980, Wyatt 1983, Webb 1999).

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菅原 敬^a, 田中伸幸^b, 邑田 仁^c: ミャンマー産 *Morinda villosa* Hook. f. (アカネ科) の二型花柱性に由来する雌雄異株性

ヤエヤマアオキ属 *Morinda* L. は、旧世界の熱帯や亜熱帯地域に分布し、80 種以上が報告されている。これらの種の性表現については、単型の両全性 (Monomorphic hermaphrodite)、二型花柱性の両全性 (Distylous hermaphrodite)、雌雄異株性 (Dioecy)、雄性両全性異株 (Androdioecy) などが報告され、繁殖システムの進化という面からも興味深い植物である。しかし、その実態については依然不明な種が多い。数年前、ミャンマー北部フーコンバレーでのフロラ調査の際、この属の 1 種 *M. villosa* Hook. f. の種子を採集する機会があり、播種したところ、今年数個体が開花した。その花形態を解析したところ、花柱と雄しべの高さが異なる二型の花が存在し、形態的には二型花柱性 (Distylous) と見なしうる。長花柱

花では雄しべは花粉を形成せず、果実をつくるのみである。一方、短花柱花では花柱柱頭の乳頭状突起が発達せず、花粉を生産するだけである。したがって機能的には完全に雌雄異株になっていることが判明した。雌雄異株性は日本に分布するハナガサノキ *M. umbellata* L. subsp. *umbellata* においても同様に見られるが、この植物の雄花では花柱が完全に消失している。一方、*M. villosa* の雄花では、短い花柱をはっきり残していることが大きな特徴である。*M. villosa* の雌雄異株性は、いくつかの属で報告されている例と同じように、二型花柱性から由来したと考えられる。これは本属における初めての例である。

^a 首都大学東京大学院理学研究科牧野標本館,

^b 高知県立牧野植物園,

^c 東京大学大学院理学系研究科附属植物園)